THE MORPHOLOGY OF ARAUCARIA BRASILIENSIS

II. THE OVULATE CONE AND FEMALE GAMETOPHYTE

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(WITH PLATES XXV–XXVII AND TWO FIGURES)

In a previous paper (1), the writer has described the source of the materials used in this investigation and the methods used in their preparation. Nothing need be added to the details presented in that paper except to mention the fact that there have been wide variations of weather conditions here in the last four years during which these collections have been made. Considerable differences have been noted in the stages reached at the same dates during these years. The 1910 collections appear to be two weeks or more farther advanced than those of the next year. It is not easy to be sure of the facts in regard to this, for cones from the same tree taken on the same day often vary astonishingly. It may be, consequently, that the variations observed are purely fortuitous and would not be sustained if the observations were sufficiently numerous, although I am inclined to think they would be. If so, it would appear that a wet winter is decidedly favorable to early development. The collections of ovulate cones were made about once a week throughout three years for most of the seasons, but were made every day or two during the months of March and April.

Each ovulate cone is borne at the end of a short branch. From three to five such branches commonly occur at a single whorl of branches (text fig. 1). The rudiments of these cone-bearing branches and that of the central leafy shoot are formed within the terminal bud of a branch. They can be found by dissecting such a bud from which the daughter buds are beginning to emerge in early April or late March. I was unable to find any recognizable trace of them earlier. Although buds exist within such terminal buds earlier, I did not succeed in finding any means of distinguishing between cones and ordinary leaf buds until just before the swellings of the ovules make their first appearance.

The buds all look alike externally before this, and even on dissection are so much alike as not to be distinguishable. A branch that has borne a cluster of cones one year does not ordinarily

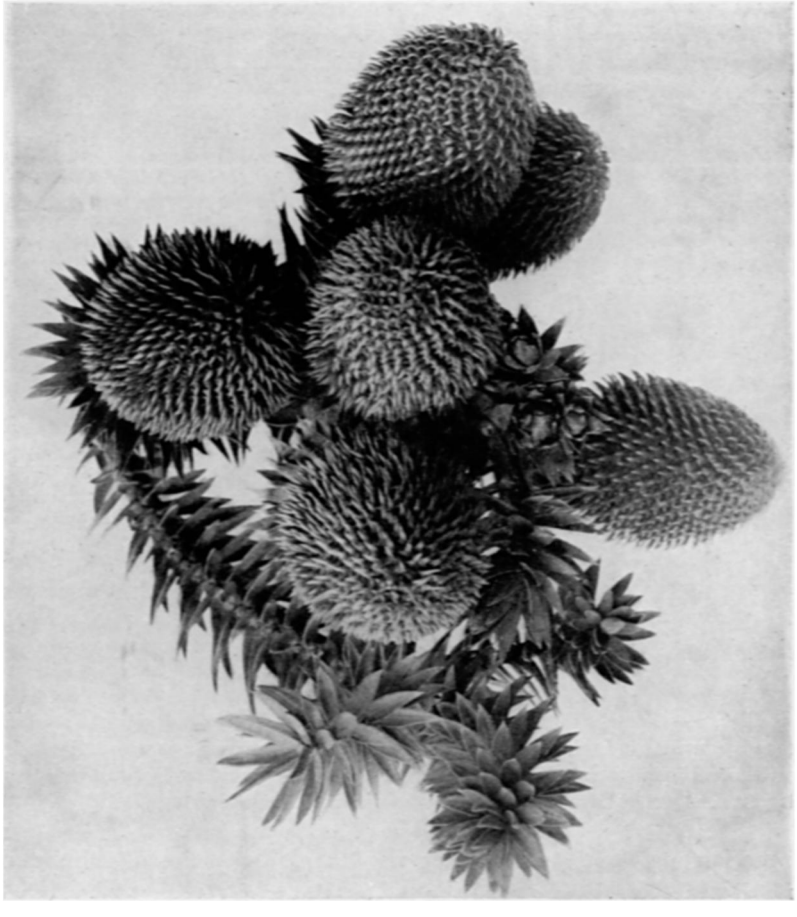

FIG. 1.—The tip of a fruiting branch bearing 6 young cones about 4 months old: the photograph was made about August 1; 4 of the 6 cones are borne on branches arising from the same whorl, the other 2 from the whorl below; the whorl just out of the bud consists of leafy branches only; $\times\frac{1}{3}$.

bear a crop the next season. A cone-bearing branch is usually thicker and looks more vigorous than a leafy branch in the spring.

When the cones have emerged from the terminal bud and are

clearly recognizable as such, they are about the size of an English walnut. They are now distinguishable from a leaf bud by their shape, by their lighter color, and by the more numerous and slenderer leaflike sporophylls. Pollination occurs at this stage. In 4 months they have reached the stage shown in text fig. 2. They are then 6–7 cm. long and about 5 cm. in diameter. The

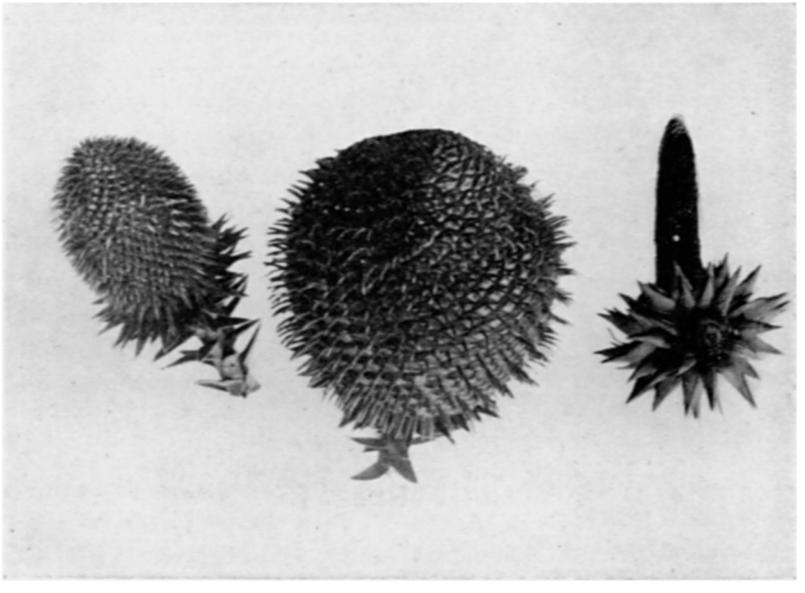

FIG. 2.—Cones of three different seasons, photographed in August: the smallest cone is 4 months old; the large cone is 16 months old; the cone axis with a few scales at base, 28 months old; the last shed its seeds in the preceding December and the largest cone would have shed seeds in the following December; $\times \frac{5}{14}$.

cone is rough and prickly from the turned-back tips of the sporophylls. The seeds are shed the fall or winter of the next year, when the cone has reached a length of 12–18 cm. and has shed many of its prickles. The life of an ovulate cone is thus somewhat less than two years. This is about a year less than that reported for *Agathis* (3), and also less than that of most conifers. There are usually 400–500 sporophylls on a cone (text fig. 2). They are arranged in steep spirals, making rather more than 1.5 complete turns.

Very soon after the cone is externally recognizable, the sporophylls show the first signs of the meristem that forms the ovule. At this time the sporophyll differs from a leaf in being differentiated into two regions. The outer part is slender and leaflike, while the basal portion is colorless, short, and stout, and tapers back slightly. The meristem is developed on the adaxial surface in a median position close to the base. It consists of many cells and grows rapidly. Figs. 1–3 show various views of it about this time. Although growth takes place throughout the sporophyll, yet four distinguishable meristems are established that determine its ultimate shape and form. The first of these is the meristem of the nucellus, already mentioned. As soon as it has made a beginning, another secondary meristem arises as a curved band across its upper surface. This curved meristematic band has its convex side directed toward the cone axis. By its growth is produced that part of the integument free from the scale. It is continuous below with the meristematic region of the scale located at its base. The fourth meristem is also a curved band, with its convexity directed toward the tip of the scale. It produces the ligule.

Not more than one sporophyll in twenty is fertile. Of those that do form ovules, many fail to mature them. Whether this tendency to sterility is equally marked in the native habitat of the tree I have not yet ascertained. It seems not unlikely that it may be due to the effects of cultivation in an alien habitat differing considerably from that of the highlands of Brazil. It has nothing to do with pollination, I am convinced, for all the cones are abundantly pollinated, both sterile and fertile sporophylls alike. The pollen tubes develop on the sterile ones, at least up to a length of 0.5 cm. or more.

The meristems very quickly produce the ovular structures. On account of this fact, and the further fact that one cannot distinguish sterile from fertile sporophylls until after sectioning them, I did not secure a complete series showing the development of the ovule. The outlines of the process, however, are clear. The basal meristem of the sporophyll elongates it, and the integument keeps pace. The result is a deep tubular cavity, deepest on the side next the sporophyll. At the base of this the nucellar meristem has been

elongating it to keep pace with the development of the integument. The result is shown in fig. 6. The line of union between the integument and the sporophyll proper is clearly indicated. In being attached along the entire side to the sporophyll, the ovule of *Araucaria* presents a sharp contrast to that of *Agathis*, which is attached only at the base (6).

In the nucellus three regions are at this time distinguishable. The tip consists of large clear cells, more or less isodiametric. Below this region the cells are elongated in the direction of the axis of the nucellus and arranged in fairly distinct rows. The rows become less definite toward the base. In the central portion of the base of the nucellus lies a group of cells with larger nuclei and denser contents. The megaspore arises in the midst of this group. This probably corresponds to the spongy tissue of various conifers, though it does not behave in precisely the same way during the further development of the ovule. Further reference to it will be made below.

The megaspore is picked out in a position above the bottom of the cleft between nucellus and integument. As it lies within the meristematic zone of the former, it follows that in the further growth of the ovule the female gametophyte will lie almost exclusively above the bottom of this same cleft. In this growth the nucellus does not enlarge its tip greatly. The result is that by the time the archegonia are ready for fertilization the nucellus is composed of a swollen base, containing the gametophyte, and a small extension above. The ovule at this time measures about 1 cm. in length and 3–4 mm. in diameter. The gametophyte extends through about half of it, and is about 1.5 mm. in diameter. It is somewhat oval in outline, with the archegonial end noticeably broader (fig. 4). The micropyle is shaped something like a human mouth, with its longer diameter transverse. Sometimes the upper lip is split back into a wide and deep V-shaped cleft extending back half the length of the gametophyte. Occasionally the latter breaks through the tissues of the nucellus and is openly exposed in the region of the archegonia. The further growth of the ovule gradually transforms the whole structure of ovule and sporophyll into the seed. The changes involved will be discussed

at another time. At maturity the gametophyte is 4–4.5 cm. long, 2 cm. wide, and 1.5 cm. thick. The entire seed structure is 5–6 cm. long, about 2.5 cm. wide, and 1.8 cm. thick.

The megaspore mother cell appears about the middle of May in the upper part of the group of denser cells already referred to. At first it differs only in size. As it enlarges, the adjacent cells show signs of disintegration. Fig. 5 shows a mother cell in synapsis. To the right and to the left of it may be seen two cells that are being flattened and whose nuclei are apparently beginning to degenerate already. I secured only a few preparations of the early stages and so cannot say certainly whether more than one megaspore mother cell ever begins development or not. Unfortunately I did not observe the reduction of the mother cell for the same reason. The chromosome numbers would indicate that a reduction does actually occur. Fig. 6 shows the position of the megaspore. In fig. 7 there may be seen two small cells just above the functional megaspore, that are probably the remains of the other members of the tetrad. A similar group is present in fig. 8. Very early the megaspore becomes vacuolate, with the very scanty cytoplasm forming an extremely delicate lining to the embryo sac. The nucleus is flattened, yet its thickness is several times that of the layer of cytoplasm in which it lies. It increases its volume, but does not immediately divide. Uninucleate embryo sacs are found in June, and binucleate ones (fig. 8) in July. By the latter part of the month as many as 64 nuclei may be present. Fig. 8 is that of a sac with two nuclei; in fig. 9 there are 8; and in fig. 10 a few less than 64. From this it would appear that the early divisions are simultaneous. As a matter of fact, I do not know that they are, for I have never observed a single mitosis in any of the more than 500 preparations of the free nuclear stages available for study. These preparations represent more than 50 collections of separate cones and two or three times as many separate ovules. It seems exceedingly strange that they should be so persistently missed. The same difficulty was encountered in a study made of the mitoses of the pollen mother cells (1). In that study I made the suggestion that those mitoses may occur at night. It has not yet been practicable to test out this hypothesis, and I mention it here merely

because I can think of nothing more likely. SAXTON (5) has since met the same difficulty and has hazarded the same guess. It may be that the mitoses are both simultaneous and passed through with extreme rapidity, and that it is merely chance that they have been missed. No indications of amitosis have been observed. Above the 64-nucleate stage the numbers are not regular, being usually somewhat less than the exact power of 2. Enlargement of the sac and multiplication of the nuclei continue up to the latter part of January. At this time there are more than 2000 free nuclei present. The cytoplasm still remains extremely scanty. Figs. 9–16 show the progress of development at intervals of about a month.

In January a change in the method of development occurs. Without any considerable increase of nuclei, the cytoplasm increases rapidly. As soon as it has become somewhat thicker, vacuoles make their appearance. The result (fig. 17) is that the central cavity is surrounded by a rapidly thickening sac of vacuolated protoplasm, with the nuclei largely confined to the inner border (fig. 18), In many cases the walls between the vacuoles break through. leaving the inner plasma membrane connected to the outer one merely by tenuous strands. The nuclei usually lie at the points where these strands join the inner plasma membrane. A few are found at the outer membrane, especially at the micropylar end of the sac. The protoplasm is thicker at this end also. This process goes forward very rapidly. The inner border advances on the central vacuole and the nuclei multiply somewhat. They now pass out along the cytoplasmic strands (figs. 19–21). By the time the inner border has closed up on the vacuole completely (fig. 20), most of the nuclei have migrated outward.

With the continued increase of the cytoplasm, most of it remains in the peripheral regions, especially near the micropylar end. It collects along the strands and plates until distinct uninucleate vacuolated sacs are formed. The nuclei are now generally suspended by still more delicate strands in the central portion of the sac. Fig. 22 shows how these sacs behave under the action of the killing reagents. Each sac appears to have its own wall of inclosing protoplasm capable of being separated from that of its neighbor. Mitoses now occur plentifully in preparations of the peripheral

portion of the prothallus. In this way there is formed a sort of colony of free cells closely packed together but yet capable of easy separation. This condition gradually passes into the vacuolated condition of the inner part of the gametophyte, in which region walls are not formed for some time. In fact, it never becomes so solid as the outer parts.

In about a month delicate walls have made their appearance between the peripheral cells (figs. 31, 32), though no definite cells have yet been organized centrally (fig. 25). The exact method of wall-formation was not made out. The process is remarkably suggestive of the way in which walls are formed in cleavage furrows in certain lower plants. One such cell is formed for each nucleus. After the formation of these first walls, the succeeding ones in the outer part are laid down on cell plates formed on the spindles in the ordinary fashion. The outer cells are generally uninucleate, while the central cells, after they have become walled off, become multinucleate by the time the archegonia are mature. They also later contain considerable quantities of starch.

In the further growth of the gametophyte, walls are formed on the spindles in the outer portion after each mitosis; in the central region wall-formation does not occur at this time. The transition from one region to the other is very gradual, and the walls thin out so gradually that it is almost or quite impossible to tell where there are actual walls. In fig. 31 are shown mitoses of both sorts. The one near the archegonium initial will have a wall formed on the spindle, while the one near the opposite border will not. Cell plates are formed in connection with the mitoses even before any walls are being formed on them (fig. 24). In the central region the nuclei are sometimes situated at the intersection of the larger strands and plates of cytoplasm and sometimes are suspended by much finer strands in the central region of the vacuolated spaces. Sooner or later these spaces, like the peripheral ones, form walls in the inclosing plates of cytoplasm and become cells. At the time of their inclosure by walls, they are commonly uninucleate. Later they frequently contain as many as 4–6 nuclei. Up to the time of the maturity of the archegonia the cytoplasm in all the cells of the gametophyte remains very scanty.

The outline of the growing prothallus may remain smooth and even (fig. 36) or become very irregular (fig. 35). The irregularity appears to be entirely due to two facts. The gametophyte, as already mentioned, is very delicate and plastic, and, in consequence, able to adapt its form to the cavity in which it grows. The second fact is that the cavity in which it is forming does not always enlarge regularly. Whether the irregularity is due in whole or in part to the action of the gametophyte is somewhat doubtful, as will be pointed out in the succeeding paragraphs. The development of the gametophyte beyond the fertilization stage will be further described in connection with the maturity of the ovule and the organization of the seed.

The cells surrounding the megaspore have already been mentioned as being larger, having larger nuclei and denser contents than other nucellar cells. They perhaps correspond to the so-called spongy tissue. The character of these cells is shown in figs. 6–8. As the megaspore enlarges, the innermost layers of these cells die and lose their contents. The walls also seem to disappear, though much more slowly. They are at first stretched out and closely compressed into a thick and compact band just outside the megaspore membrane. Inasmuch as this band of crushed cells does not appear to increase in thickness beyond a certain point, it seems reasonable to suppose that it is dissolved and perhaps used by the growing gametophyte. Immediately outside of this region of dead and empty cells there is a more or less distinct band of cells (figs. 10, 12, 13, 14) which stain more densely. This band of cells develops outward in advance of the gametophyte, keeping much the same relation to it as at first. The individual cells have more cell contents than the cells outside of the band. Their nuclei take basic stains strongly, as sometimes does the cytoplasm also. In short, they appear to be undergoing degeneration. The appearances described are such as have been observed in many other plants. The phenomena have been commonly ascribed to the effects of the gametophyte. It has been supposed that it secreted some sort of enzyme or other substance that diffused outward, killed and digested the cells, and prepared them for food for itself. It looks like a reasonable inference.

In the preceding paragraph I have described the appearance of the nucellar tissues around the growing gametophyte. I wish now to describe some of the anomalous conditions found that have led me to suspect the validity of the current accounts of the effects of the gametophyte on the nucellus. In fig. 26 is shown an apparently enlarging hole in the nucellus surrounded by the two usual bands of differentiated cells. Such ovules are fairly common. In some cases the megaspore membrane appears to be present. One might infer in such cases that the hole is the work of a gametophyte that for some reason or other has died. In most cases the megaspore membrane cannot be demonstrated; but since it is not well developed in any case, this would not appear to be an insuperable difficulty.

Fig. 27 shows a less common condition. There is no gametophyte here, nor is there any place for one; yet there is a remarkable correspondence in nucellar structure. In the center there is an enlarging mass of cells whose nuclei and cytoplasm are undergoing degeneration. The central cells are almost completely crushed; they have very little or no living contents. Outwardly the cells grade off through less and less crushed cells to a band of normal shape and size, but with densely staining contents, just as in the normal ovules. If there is no gametophyte and even no place for one, then the effects cannot be due to the presence of one. It seems that these facts admit of but one of two possible explanations: either the cells of the spongy tissue, which are possibly potential megaspore mother cells, are capable of producing the observed effects, or it is a quality of the nucellar cells themselves to behave in this fashion, regardless of the presence of a gametophyte or its antecedent archesporial tissue. A noticeable peculiarity of these cells in all cases is the thickening of their walls accompanying the death and disappearance of the protoplasm.

The development of such sterile ovules has nothing to do with pollination, apparently, for they occur regardless of whether the pollen has or has not sent tubes into the nucellus. They are relatively common and develop to advanced stages. Cones on unpollinated trees on the grounds of Stanford University develop to nearly normal size, though the gametophytes do not.

The megaspore membrane is usually thin and poorly organized.

It is variable in different ovules. In *Agathis* it is reported (3) that the megaspore membrane is thickest over the apex of the gametophyte and gradually thins out toward the archegonia in such a way as to allow the fertilization of the lower archegonia first and to protect from the pollen tubes the later maturing upper archegonia. There does not appear to be any such difference in *Araucaria*, though it is usually the case that the megaspore membrane disappears along with the band of dead cells in the region of the archegonia (fig. 4).

The time at which the pollen tubes reach the nucellus is subject to wide variations. They may do so as early as July or be deferred till late in the fall. The time at which they do so does not appear to exert any influence on the development of gametophyte or ovule, within the limits mentioned. So many tubes commonly reach and penetrate the nucellus that it is almost entirely destroyed. They usually enter through the tip, composed of large clear cells with little protoplasm, but may occasionally pass between the nucellus and integument for a very short distance before entering the former. In the cases of the large slitlike micropyles, through which the nucellus is exposed for a large part of its upper surface, the tubes ordinarily, at any rate, enter only through the tip. There do not appear to be any special peculiarities in the way the tubes penetrate the nucellus. They go fairly straight to the region of the archegonia. Occasionally one branches; a few strike the cap of dead cells over the apex of the gametophyte and then commonly turn aside. They are surrounded by a layer of dead cells somewhat like that around the female gametophyte, though it is less extensive and less regular. No indications of the breaking down of nucellar cells to make way for a pollen tube were observed unless the pollen tube was itself present to account for the effects. The nucellar cells below the tip commonly contain much starch, which largely disappears with the development of the tubes.

The uniformity with which the tubes enter the tip of the nucellus, even when a shorter and apparently more available path is present, suggests that they are attracted by a chemotactically active secretion from the glandular tip. I did not succeed in demonstrating such a secretion. There are often considerable quantities

of a slightly sticky liquid between the sporophylls, but I failed to find any evidence that it comes from the nucellar tip.

At about the time the walls are formed in the peripheral parts of the gametophyte, in the latter part of February usually, the archegonium initials become recognizable. Owing to their scanty contents they are recognizable only after they are somewhat enlarged. They vary in number from about 6 to 15 or more. They are situated in a ring around the crown of the prothallus. They do not all mature at the same time, though there does not appear to be any regular order. Commonly 5–8 mature and 3 or more of these are frequently fertilized. Within the circle the individual archegonia may stand alone (fig. 4) or they may be grouped in complexes (figs. 30, 44). Each archegonium is commonly surrounded by an individual jacket, though in some of the complexes there may be no cells at all between some of them (fig. 44).

The initial is commonly a wide U-shaped (fig. 31) or V-shaped (fig. 32) cell. It has a large nucleus and little cytoplasm. Sometimes a basal cell is cut off from this cell (fig. 33) before it becomes the actual initial. The nucleus of the initial divides and a periclinal wall separates a thin flat primary neck cell from the inner or central cell (figs. 32, 34, 37). The central cell enlarges much more rapidly than the neck cell (figs. 38–40). The latter soon divides by an anticlinal wall. This division is more frequently in the direction of its greater diameter. Each of the halves then divides into about 6 wedge-shaped cells. The nuclei of these wedge-shaped cells are invariably at the large end of the wedge. The points of the cells meet or nearly meet at the center of the neck. At this point the cells have commonly thinner walls, less cytoplasm, and a tendency to separate and leave a free passage to the egg (fig. 46). Viewed from above, the group of neck cells has either a rounded (fig. 46) or elliptic (fig. 47) outline; from the side they usually appear as a dome or cap (fig. 45).

Many variations in the form and outline of the neck occur. The size of the group as well as the number of cells in it is subject to considerable variation. The commonest arrangement is 12 cells arranged in a single tier (figs. 45, 46). Figs. 48–50 show three serial sections through a neck in which there is one extremely large cell.

The figures also show that the cells are not all in the same plane, but are placed more or less obliquely above one another. This is not unlikely due to crowding by the large cell. Many such asymmetrical necks occur in my preparations. Occasionally one cell is so large as almost to pass for a central cell. These large neck cells possess large nuclei, as may be seen from fig. 50. Occasionally there is more than a single tier of cells in the neck; fig. 51 shows a neck of 3 tiers of cells.

All the archegonial initials which I could certainly identify and all young archegonia occur in the surface layer of cells. In one preparation there were 4 cells in a row. The outer one resembled a primary neck cell. The lowest one was large and had the general appearance of a central cell. The inner of the other two was about one-third as large as the lowest and slightly larger than the second one. An imbedded archegonium might have resulted, possibly, from such an initial as this. Though the mature archegonia are very frequently displaced and overgrown by the neighboring cells, it is ordinarily easy to find the free open passages from them to the surface. I did not find any case in which it was not very probable that such a passage exists. I am fairly convinced, therefore, that there are no deep-seated archegonia in this species of *Araucaria*. I believe that all such appearances are due to displacement and overgrowth. EAMES has recently (3) expressed a somewhat similar opinion in regard to *Agathis*. I have examined a number of preparations of *A. imbricata* and have seen no deep-seated archegonia among them. The displacement and overgrowth of the archegonia is rendered very easy owing to the very delicate walls in the prothallus and to its frequently irregular outline. The more solid archegonium would thus be easily pushed into a position where the turgescent cells of the gametophyte would find an equilibrium of mutual pressures. The same fact would almost invariably lead to the crowding down into the archegonial cavity of the adjacent cells if they encountered any resistance at all in the expansion of the cavity in which the gametophyte grows.

The jacket usually consists of a single layer (figs. 42, 43) of more dense cells, which are usually uninucleate and with comparatively thin walls. The wall next the egg is somewhat thicker than the

others and is usually marked by a large thin spot, as is common among conifers. Occasional cells are binucleate, and sometimes the jacket is doubled in places (figs. 49, 50). In contrast to *Agathis* (3), the jacket is firmly united to the neck cells. In consequence of this the contents of the pollen tube pass through between the neck cells, in a manner to be described in another place.

The growth and development of the central cell resembles that of most other conifers in its general outlines. It enlarges rapidly but remains poor in cytoplasm (figs. 37–40). At first there is a single large vacuole, later there are many small ones in the more abundant cytoplasm (figs. 41, 42). At maturity there are usually no vacuoles and the cytoplasm is very dense (fig. 43). The nucleus enlarges with the development of the central cell. At first it is placed in the upper central part (figs. 39–41). It later migrates to one side (fig. 42) and nearer the neck. From here it passes to a position just below the neck (fig. 45) as if about to divide into egg nucleus and ventral canal nucleus. I was unable to find any evidence that it does divide. No mitotic figures were seen in the developing archegonium, nor were any nuclei, other than the one, ever seen before fertilization, except in one ovule. In this case a number of small nuclei were present in the upper part of what appeared to be a mature archegonium as yet unattacked by a pollen tube. It would be rash, perhaps, to assert that such a ventral canal nucleus is never cut off, even though a persistent hunt for it has failed to reveal it.

Discussion

I shall not attempt at this time to discuss broadly the relationships of *Araucaria* to other members of the Coniferales, but merely to point out wherein it resembles some of them and in what ways it differs from all of them in certain features of its ovule and female gametophyte.

One of the first points in which this species of *Araucaria* (also *A. imbricata*) differs from *Agathis* is in the length of time taken to mature seeds from the first appearance of the seed cone. From the time it can be first recognized until the seeds fall is approximately 21 months. EAMES (3) reports *Agathis* as forming the

rudiments of its seed cones nearly a year in advance of pollination, while here there intervenes scarcely any time at all. In fact, in California the greater part of the pollen is likely to be shed before there are any ovulate cones to pollinate. Observations in the native habitat might show a different state of affairs and one more nearly paralleling that of *Agathis*. Other conifers of course are known in which the total time is even shorter than 21 months, but I am not aware of any one in which the time is distributed in the same manner.

The very considerable number of free nuclei before cell formation is another character that, taken with the very large gametophyte, reminds one of more primitive gymnosperms. Gametophytes of this size are known only among the Cycadales, Ginkgoales, and some taxads (as *Torreya*). A curious feature of its development is the strange absence of mitoses in the free nuclear preparations.

The manner in which the free nuclear stage passes into a gametophyte with walled cells is apparently different from that reported for any other plant. While there is a certain resemblance to the centripetal growth with "alveoli" reported for *Sequoia* (4) and others, yet the exact method is really quite different. The most essential feature of this difference lies in the delay of walls. If walls formed between the nuclei before the beginning of the centripetal movement of the inner border of the cytoplasm and were extended *pari passu* with it, and the nuclei divided to keep pace, there would be no very essential difference. While far too little is known about the exact methods of centripetal growth and wall-formation in any considerable number of genera to make any conclusions based on this feature more than tentative, it is clear that *Araucaria* need not be excluded from relationship either with podocarps or with abietineous conifers on this account.

While there is no proof that the gametophyte invades the nucellus in *Araucaria*, neither is it proved that it does so in other genera where similar appearances are commonly observed. The homology of the spongy tissue and its functions is none too clear anywhere. The peculiar glandular nucellar tip is found elsewhere only among conifers of podocarpineous affinities. THOMPSON has made the suggestion that the method of pollination found in the

araucarians is "proto-angiospermic." It seems to me, on the contrary, that we have been altogether too ready to accept the type of ovule which has a specialized pollen chamber securely hidden away at the base of the scale, and which can be reached by pollen only by means of special devices, as a primitive type. It is scarcely credible that the first step in the evolution of the ovule and seed should have been so complex. If, however, the gymnosperms possessing this complex type evolved a seed before a cone, as in fact there is good reason to think they did, then this might have been at least one of the earlier successful types. If, on the contrary, the cone was evolved before the seed, or simultaneously with it, as it may very well have been in an apparently simple cone, what would be more natural than that the pollen should lodge in any convenient place among the scales (sporophylls perhaps) of the cone? Such an ovule would have a much better chance of survival in such a cone than if exposed on the lobe of a fernlike leaf such as those possessed by the known Cycadofilicales. I am aware that I am thus attempting to introduce an apparently ancestorless conifer, but fail to see that a search for fitting ancestors is likely to be more difficult than deriving it from unsuitable ones.

Imbedded archegonia have been reported (6) for *Araucaria*, and a comparison made with *Sequoia* in which somewhat similar conditions are said to be present (4). My own observations do not bear out the presence of such imbedded archegonia in *Araucaria*. They are superficial in origin and become overgrown by the neighboring cells. SINNOTT has recently reported (7) practically identical conditions in the podocarps. The necks are not specially noteworthy, though they show a rather closer resemblance to those reported for *Podocarpus* (7) than to those of most other conifers.

The failure to find a ventral canal nucleus is somewhat surprising in so large a gametophyte, not having advanced beyond the evolutionary stage in other respects that has been attained by conifers generally. It seems more probable that it will yet be found. Ventral nuclei have not been found as yet in one species of *Torreya* (2).

The gametophyte, therefore, appears to be neither highly specialized nor exceptionally primitive in its structure. Its large

size and numerous and large archegonia are offset by the late development of walls and their persistent delicacy, by the apparent lack of a ventral canal cell, and by the rather specialized necks of the archegonia. Probably it presents more resemblances to the gametophytes of the Taxaceae and to those of the Taxodineae than to other conifers.

Summary

1. The ovule possesses a very free nucellus with a glandular tip, a single integument adherent to the scale for almost its entire length, a ligule, a large micropyle, and spongy tissue surrounding the gametophyte.

2. There is probably a single functional megaspore, which develops into an embryo sac with about 2000 free nuclei before cell-formation.

3. Cell-formation follows on a peculiar centripetal growth of the cytoplasm and precedes wall-formation.

4. The first walls are formed on the surface of the free cells.

5. Secondary walls are formed on the spindles of the mitoses occurring in the primary cells of the peripheral regions of the gametophyte.

6. The outer cells are uninucleate, the inner ones are multinucleate.

7. The archegonia have single-tiered necks, usually, consisting of about 12 wedge-shaped cells.

8. The necks are on the surface of the prothallus but are often overgrown.

9. The archegonia may be single or occur in complexes and have a single-layered jacket.

10. A ventral canal nucleus may be absent.

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EXPLANATION OF PLATES XXV–XXVII

FIG. 1.—Longitudinal section of a young cone in which the rudiments of the ovule are just becoming visible; ×12.

FIG. 2.—Longitudinal section of a slightly older sporophyll, showing the beginnings of the nucellus, integument, and ligule; ×15.

FIG. 3.—Longitudinal section through the meristem of the nucellus; ×62.

FIG. 4.—Longitudinal section of an ovule just before fertilization, showing the position and relative size of the gametophyte and a protruding nucellus; ×10.

FIG. 5.—Megaspore mother cell in synapsis; ×900.

FIG. 6.—Longitudinal section through an ovule in the latter part of June, showing the enlarging megaspore; ×20.

FIG. 7.—A megaspore just before division, surrounded by spongy tissue; ×62.

FIG. 8.—A binucleate embryo sac in late June; ×62.

FIG. 9.—An 8-nucleate sac in July; ×62.

FIG. 10.—A 64-nucleate stage in late July; ×62.

FIG. 11.—A 512-nucleate stage in late August; ×20.

FIG. 12.—A small portion of the parietal protoplasm and nuclei in late October; ×125.

FIG. 13.—A whole embryo sac in October; ×20.

FIG. 14.—An embryo sac in November; ×20.

FIG. 15.—An embryo sac in December; ×20.

FIG. 16.—An embryo sac in January; ×20.

FIG. 17.—The beginning of centripetal growth of the cytoplasm; ×20.

FIG. 18.—The micropylar end of the gametophyte shown in fig. 17; ×125.

FIG. 19.—Centripetal growth half complete; note that some of the nuclei are now migrating outward and that there are no indications of walls; ×20.

FIG. 20.—A detail of the same gametophyte as shown in preceding figure; ×62.

FIG. 21.—The completion of centripetal growth of the cytoplasm; ×62.

FIG. 22.—Detail from micropylar end of sac of age shown in preceding figure; ×125.

FIG. 23.—A slightly older gametophyte; ×20.

FIG. 24.—A detail from preceding figure showing lack of walls and presence of free cells; ×250.

FIG. 25.—A central part of the gametophyte in early March, showing the lack of walls; from the same slide as fig. 31; ×125.

FIG. 26.—Section through an ovule in which the nucellus is growing and the cavity enlarging, but in which there is no gametophyte; ×20.

FIG. 27.—Section through an ovule without any gametophyte, but with its place occupied by an enlarging mass of cells probably derived from the spongy tissue; ×20.

FIG. 28.—Early stage of the erosion of nucellus by the pollen tubes, July; note the abundant starch; ×62.

FIG. 29.—A nucellus in December when most of the starch has disappeared and all the upper part of the nucellus has been destroyed by the tubes; ×62.

FIG. 30.—Section through an archegonial complex; note the remains of the apical cap of dead cells and the superficial neck; ×85.

FIG. 31.—Micropylar end of thallus about March 1, with archegonial initial; ×125.

FIG. 32.—Lateral portion of another thallus with young archegonium near top; ×125.

FIG. 33.—An archegonium initial just before division; ×250.

FIG. 34.—Young archegonium just after cutting off primary neck cell; ×250.

FIG. 35.—Apical end of an irregular thallus with young archegonia; ×62.

FIG. 36.—Similar thallus with smooth outline and young archegonia; ×62.

FIG. 37.—Young archegonium; neck cell undivided; ×250.

FIG. 38.—Similar archegonium with neck divided; ×250.

Figs. 39, 40.—Enlarging archegonia; ×250.

FIG. 41.—Archegonium about one-half mature, showing neck, vacuolate cytoplasm, jacket, and position of nucleus; ×125.

FIG. 42.—Nearly mature archegonium with nucleus to one side and just below neck; ×125.

FIG. 43.—Mature archegonium; ×125.

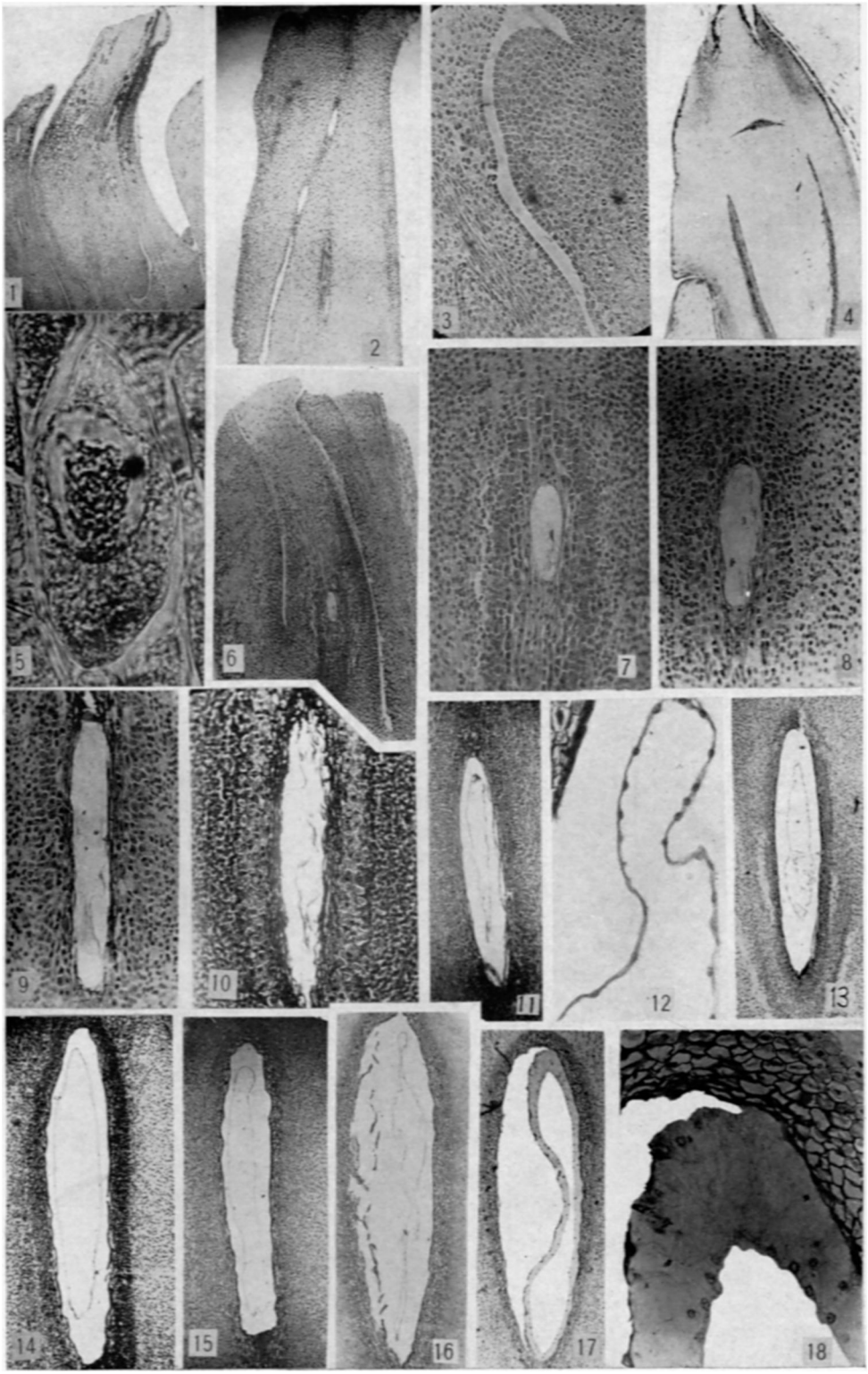
FIG. 44.—Cross-section through a complex where there are no jacket cells between two of the archegonia; ×125.

FIG. 45.—Section showing dome-shaped neck and position of nucleus below it; ×250.

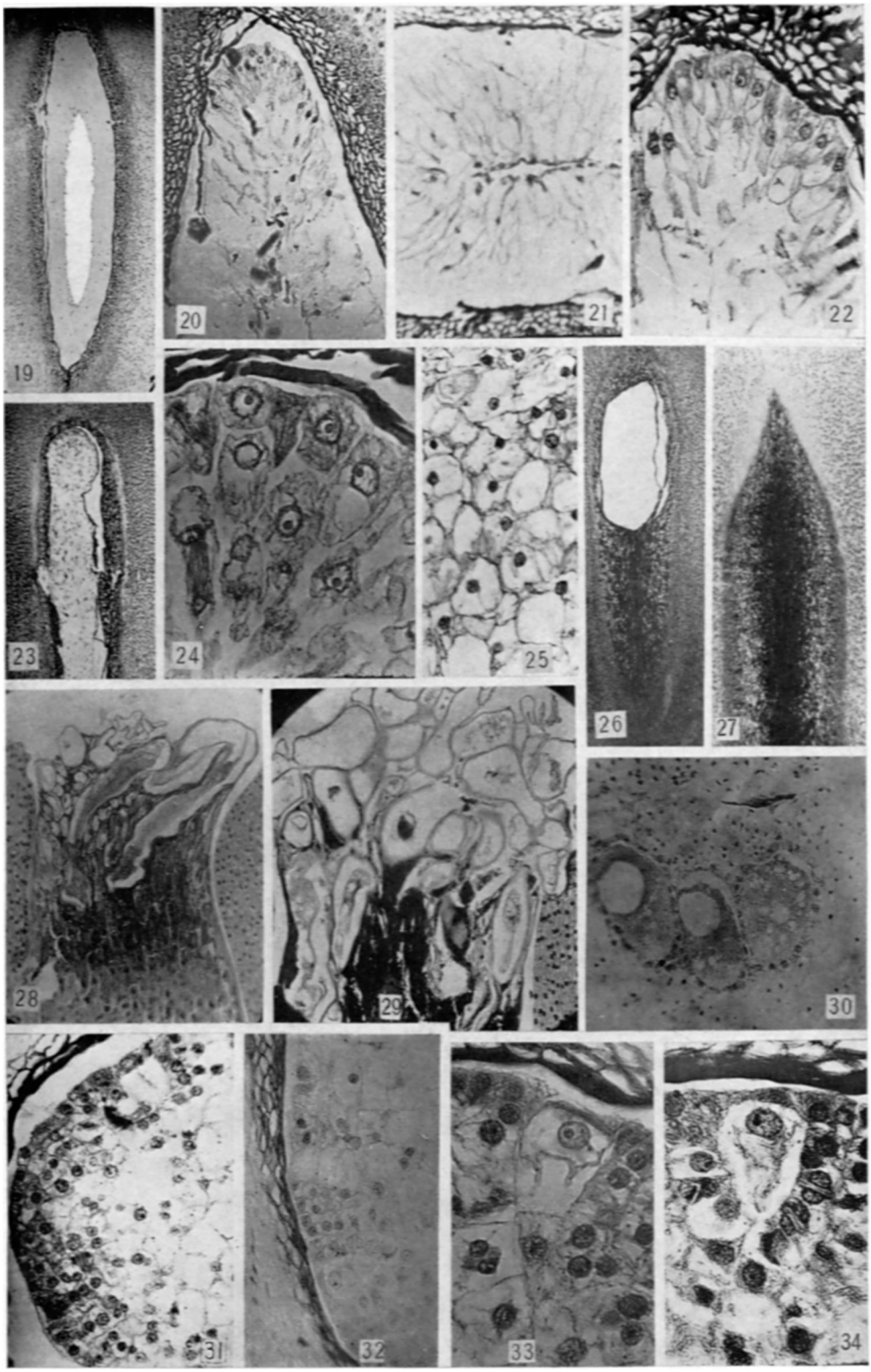
FIGS. 46, 47.—Cross-sections through usual type of neck; ×250.

FIGS. 48–50.—Serial sections through neck with one very large cell; ×125.

FIG. 51.—Longitudinal section of 3-tiered neck; ×250.



BURLINGAME on ARAUCARIA BRASILIENSIS



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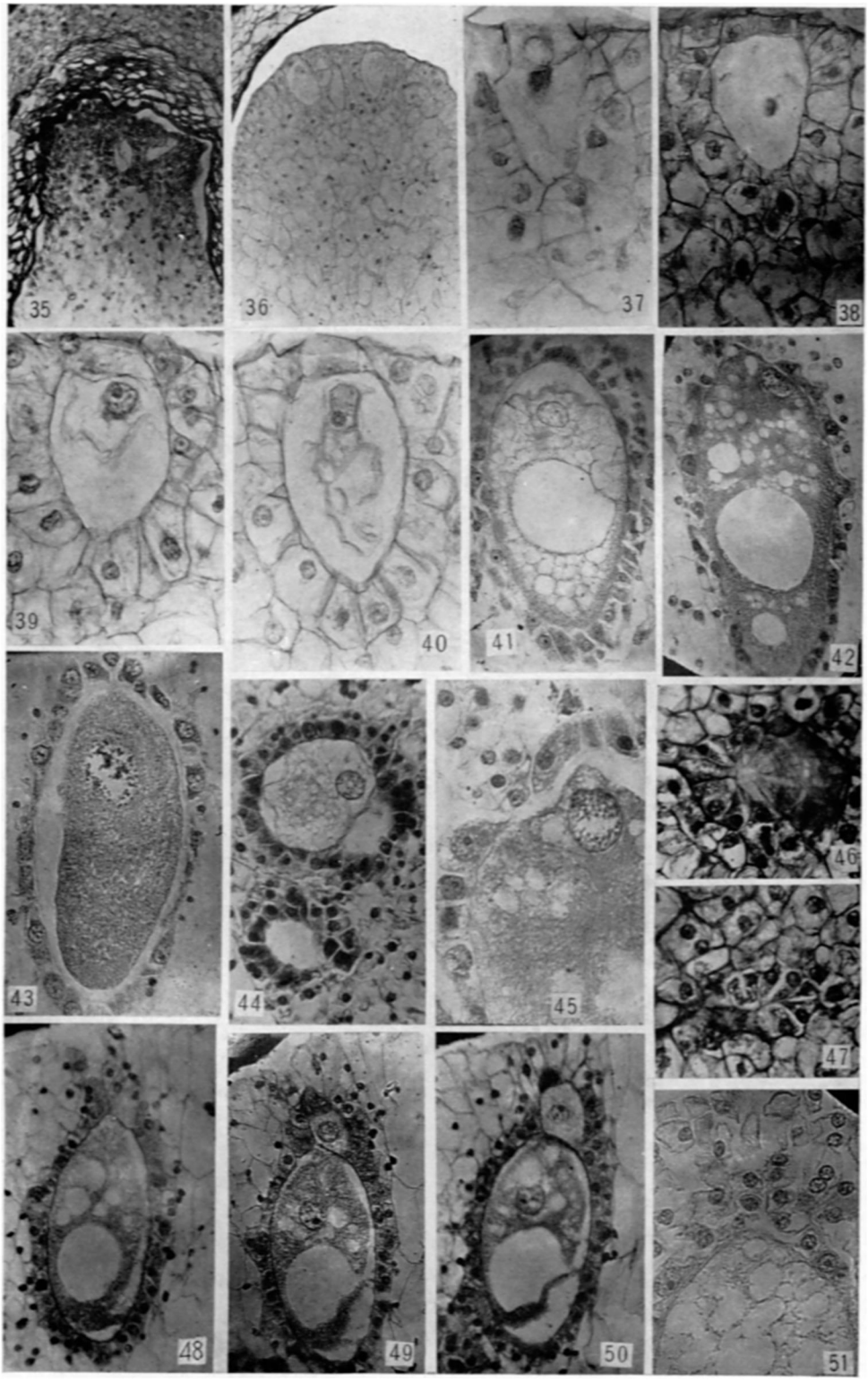

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